REMARKS/ARGUMENTS

Claims 60 and 64-66 are active. Claims 1-33 have been withdrawn from consideration. Claims 60 and 64 have been amended to appear in independent form. Claim 60 finds support in the nucleic acid which encodes a biosensor protein of Probe No. G18, G72 or G85 listed in TABLES 3 and 4 (see the specification, pages 48-49). Claim 64 finds support in a nucleic acid molecule encoding a biosensor protein of Probe No. A1 or A2 listed in TABLE 5 (see the specification, page 54). Claim 65 finds support in a nucleic acid molecule encoding a biosensor protein of Probe No. G3, G4, G17, G18, G47, G52, G54, G61, G72, G76, G77, G79, G80, G81 or G85 listed in TABLES 1, 3 and 4 (see the specification, pages 39 and 48-49). Claim 66 finds support in a nucleic acid molecule encoding a biosensor protein of Probe No. G3, G4, G13, G17, G18, G22, G23, G27, G47, G52, G54, G61, G72, G76, G77, G79, G80, G81 or G85 listed in TABLES 1 to 4 (see the specification, pages 39-40 and 48-49). Calmodulin protein mutant "CaMCN" recited by the claims is described on page 43, lines 10-13 of the specification. Attached to this response is a copy of Persechini A et al., 1997, Cell Calcium 22, 209-216 incorporated to describe this protein on page 43, lines 12-13 of the specification. Page 209, "Abbreviations" describes that "CaMCN" is an engineered CaM (calmodulin) in which residues 82-148 and 9-75 have been exchanged. Page 210, "Materials and Methods" indicates that "in CaMCN the native C- and N- terminal EF hand pairs are exchanged". Accordingly, the Applicants do not believe that any new matter has been added. Favorable consideration of the amendment is now respectfully requested.

Election/Restriction

The Applicants hereby affirm their election with traverse of **Group III, Claims 34-64**. The traverse is on the grounds that the subject matter of each group is substantially interrelated and that no undue burden would be imposed in the examination of Claims 1-64 together. In the event that the restriction requirement is maintained, the Applicants respectfully request that the claims of the nonelected groups which depend from or include all the limitations of those of Group III, be rejoined upon an indication of allowability for the elected claims, see MPEP 821.04.

Objection to the Specification and Claims—Sequence Rules

The Preliminary Amendment filed February 5, 2003, previously added sequence identifiers to pages 10, 12, 19, 20, 37, 42, 43, 50, 51, 62, 63 and 64 of the specification. However, page 47 of the specification is amended herewith to add the appropriate SEQ ID NO. The claims have also been revised to refer to specific sequence identifiers. Accordingly, the Applicants submit that this rejection may now be withdrawn.

Objection

Claims 34-64 were objected to as being in improper form. This objection is moot in view of the above amendment.

Rejection—35 U.S.C. 112, second paragraph

Claims 34-64 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite. These rejections are moot in view of the amendments above.

Rejections—35 U.S.C. 102

Claims 34-46, 47-59 and 61 were rejected under 35 U.S.C. 102(e) as being anticipated by Persechini, U.S. Patent No. 6,376,257 or Baird, PNAS 96:11241. These rejections are moot in view of the cancellation of these claims. Moreover the Applicants submit that these documents would not anticipate or render obvious the present invention for the following reasons.

Persechini discloses a biosensor protein that includes two green fluorescent proteins (namely, a first green fluorescent protein and a second green fluorescent protein), and a calmodulin-binding peptide--see FIG. 1 and Claim 9 of Persechini. Thus, the biosensor protein of Persechini has two green fluorescent proteins (GFPs), none of which is modified.

By contrast, as recited in the amended claims, the biosensor protein encoded by the nucleic acid molecule of the present invention has one GFP molecule, which is modified--see the specification, FIG. 1 Band FIG. 1 D. As described above, Persechini does not disclose or even suggest a biosensor protein encoded by the nucleic acid molecule of the present invention, that is, a biosensor protein having a modified GFP molecule.

The biosensor protein disclosed in Baird et al. has such a structure that calmodulin is inserted into Tyr-145 of the yellow mutant (EYFP) of GFP. More specifically, the biosensor protein disclosed in Baird et al, has a sequence indicated in FIG. 1(d) on page 11243, in which the amino acid sequence from No. 1 to 144 of EYFP, calmodulin, and the amino acid sequence from No. 146 to 238 of EYFP are arranged in this order from the N terminal.

By contrast, the biosensor protein encoded by the present invention, recited in the amended claims 60 and 65 includes a modified GFP that has the "amino acid

sequence from No. 149 to 238 of GFP" and the "amino acid sequence from No. 1 to 144 of GFP" in the order from the N terminal. The biosensor protein encoded by the present invention, recited in the amended claim 66 includes a modified GFP that has the "amino acid sequence from No. X to 238 of GFP (where X is any one of 148, 149 and 150)" and the "amino acid sequence from No. 1 to Y of GFP (where when X is 148, Y is 140, when X is 149, Y is 144 or 147, or when X is 150, Y is 144 or 14T, in the order from the N terminal. The biosensor protein encoded by the present invention, recited in the amended claims 64 includes a modified GFP that has the "amino acid sequence from No. 1 to 144 of GFP" and the "amino acid sequence from No. 149 to 238 of GFP" in the order from the N terminal.

As discussed above, the biosensor protein of Baird et al. has such a structure that the yellow mutant (EYFP) of GFP is modified, whereas the biosensor protein of the present invention has a structure in which GFP is modified. In this respect, these structures are different from each other. In addition, the biosensor proteins of Baird et al. and the present invention have different amino acid sequences in the modified fluorescent proteins as described above. Thus, Baird et al. does not disclose or even suggest a structure similar to that of the biosensor protein encoded by the nucleic acid molecule of the present invention. Therefore, it is only natural that the biosensor of the present invention, in which the modified GFP and the specific functional proteins are linked in the specific order by the specific linkers as recited in the amended claims, is not disclosed or even suggested at all in Baird et al.

Further, Baird et al. (page 11244, right column, lines 14 to 17) discusses that, as a sensor, such a large response to Gal+ as that of EYFP cannot be obtained when GFP is used. Thus, this document may suggest the use of EYFP for producing a biosensor protein, but does not suggest the use of GFP. In this manner, Baird et al,

suggests an opposite manner to that of the present invention in producing a biosensor with use of a fluorescent protein.

Moreover, the biosensor protein encoded by the nucleic acid molecule of the present invention has the following excellent advantages, as compared to Baird et al. That is, the biosensor protein of the present invention has such a high sensitivity that if there is only an extremely small amount of target substance to be detected by the biosensor, it can be surely detected. Further, the biosensor protein has such a high responsiveness that the change even in an extremely small amount of a substance to be detected can be reflected in the fluorescence property of the biosensor. In detail, the biosensor protein of the present invention has, as indicated by the dose-response curve presented in the specification, FIG. 5, a property where Kd = 285 nM, whereas Baird et al. has, as shown in Fig. 4c, a property where Kd = 7μ M. Thus, the biosensor of the present invention has a sensitivity of about 30 times higher than that of Baird et al. In addition, the biosensor protein of the present invention has, as indicated in the specification, FIG. 5, a Hill coefficient of 3.23, whereas Baird et al. has a Hill coefficient of only 1.6. Thus, the biosensor of the present invention has a superior responsiveness to that of Baird et al. Accordingly, the Applicants respectfully request that these rejections now be withdrawn.

CONCLUSION

The Applicants respectfully submit that this application is now in condition for allowance. Early notification to that effect is earnestly solicited.

Respectfully submitted,

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